

## Feature

## Oil biodegradation and bioremediation: A tale of the two worst spills in U. S. history

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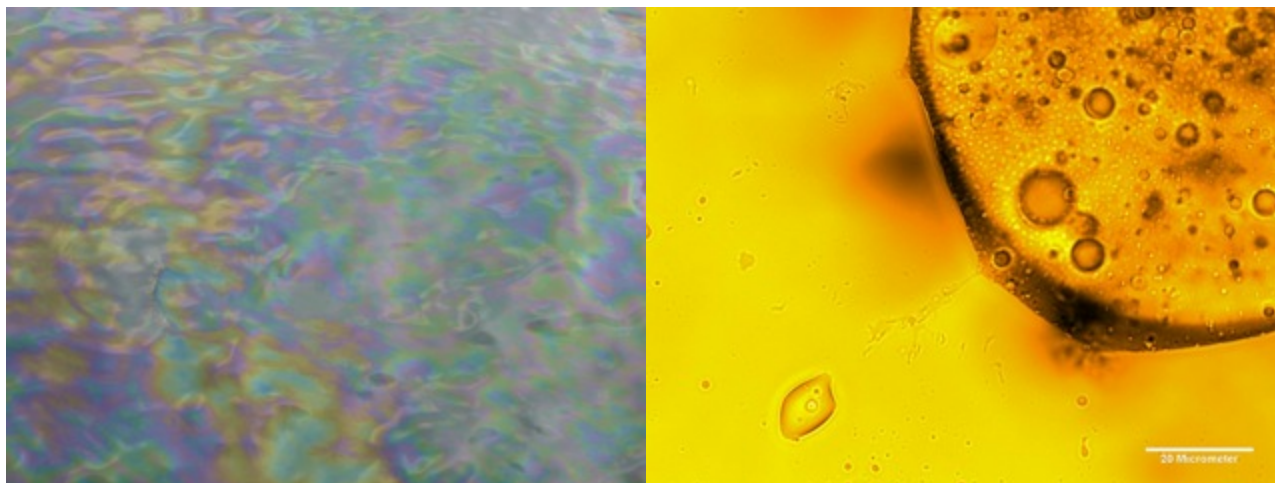
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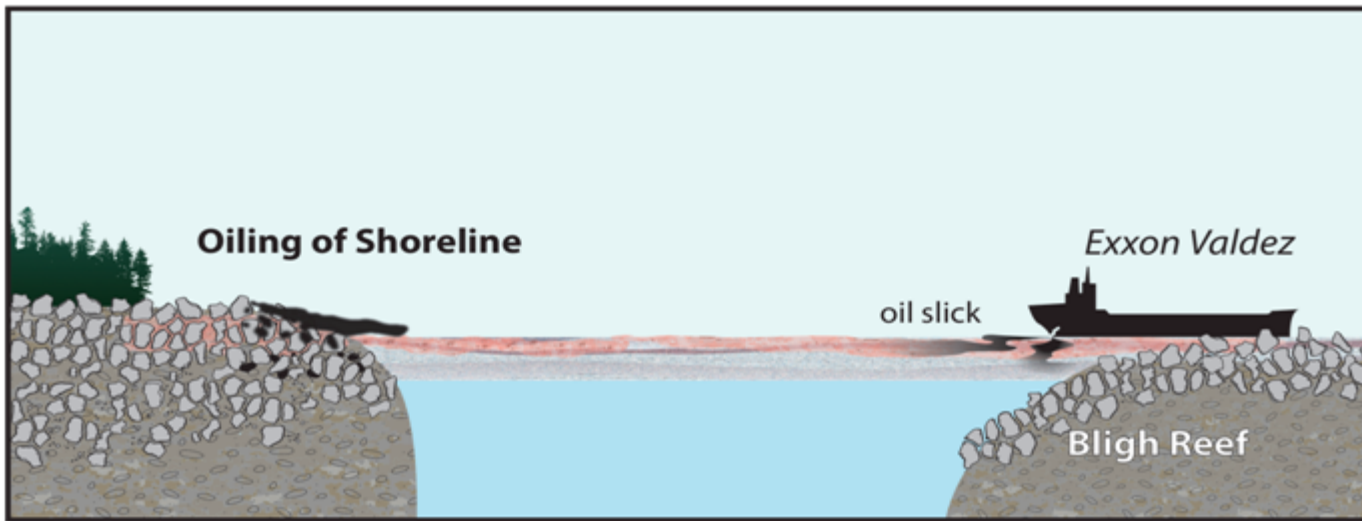
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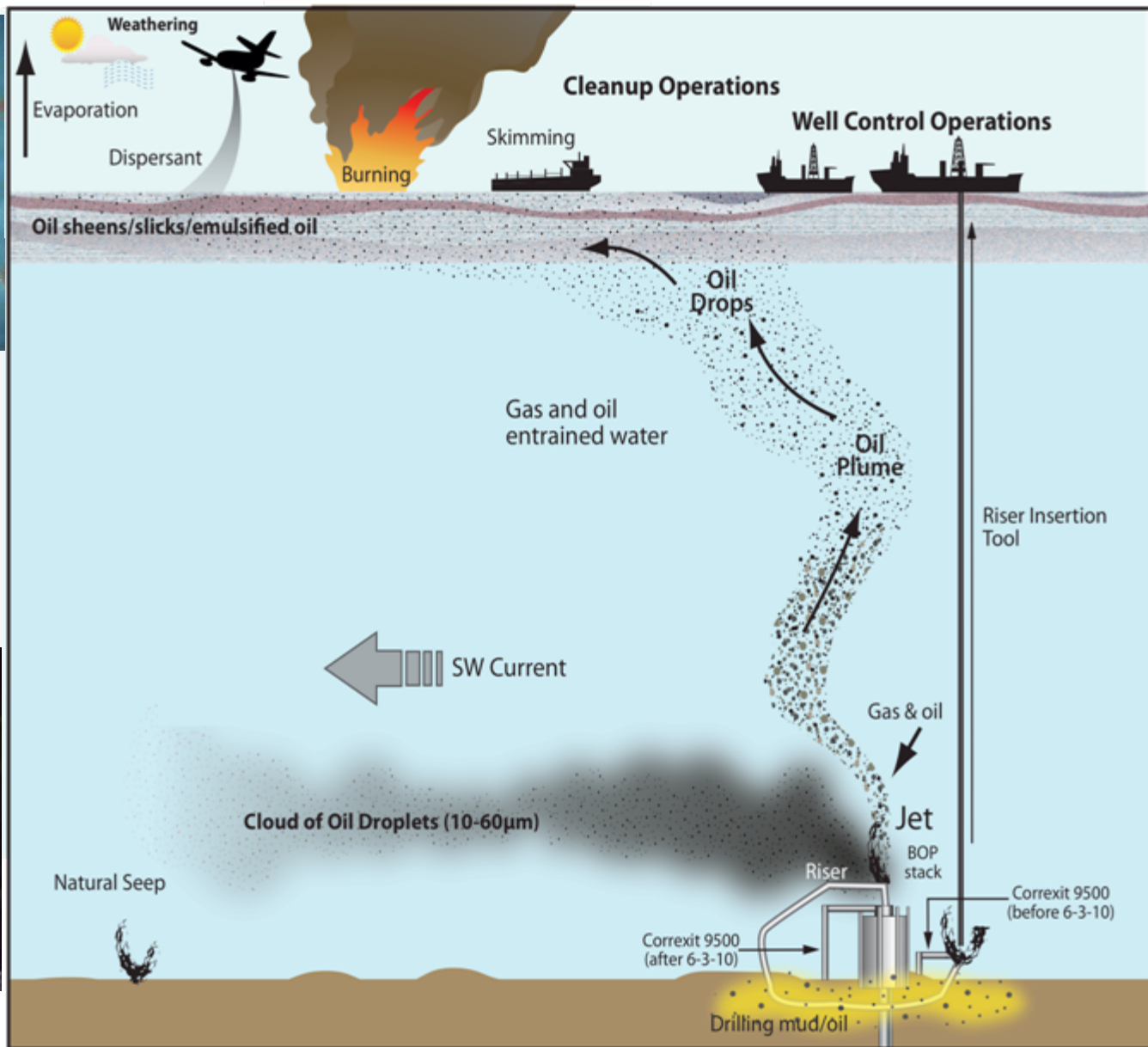




Physical washing of shorelines  
with high pressure water



Application of fertilizer for  
bioremediation



## Oil biodegradation and bioremediation: A tale of the two worst spills in U. S. history.

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### Biography

Ronald Atlas is Professor of Biology at the University of Louisville. He has over 40 years experience studying the role of microorganisms in oil biodegradation and helped pioneer the field of bioremediation. He has worked extensively on the bioremediation the Exxon Valdez spill. Terry Hazen is DOE BER distinguished scientist in the Earth Sciences Division at Lawrence Berkeley National Laboratory. He has studied oil, chlorinated solvent, and metal and radionuclide bioremediation for more than 30 years. He has been extensively studying the microbial degradation of the BP *Deepwater Horizon* Spill in the Gulf of Mexico.

### Abstract

The devastating environmental impacts of the *Exxon Valdez* spill in 1989 and its media notoriety made it a frequent comparison to the BP *Deepwater Horizon* spill in the popular press in 2010, even though the nature of the two spills and the environments impacted were vastly different. Fortunately, unlike higher organisms that are adversely impacted by oil spills, microorganisms are able to consume petroleum hydrocarbons. These oil degrading indigenous microorganisms played a significant role in reducing the overall environmental impact of both the *Exxon Valdez* and BP *Deepwater Horizon* oil spills.

### Introduction to Biodegradation of Petroleum Hydrocarbons

Petroleum hydrocarbons in crude oils, such as those released into marine ecosystems by the *Exxon Valdez* and BP *Deepwater Horizon* spills, are natural products derived from aquatic algae laid down between 180 and 85 million years ago. Crude oils, composed mostly of diverse aliphatic and aromatic hydrocarbons, regularly escape into the environment from underground reservoirs. Because petroleum hydrocarbons occur naturally in all marine environments, there has been time for numerous diverse microorganisms to evolve the capability of utilizing hydrocarbons as sources of carbon and energy for growth. Oil-degrading microorganisms are ubiquitous, but may only be a small proportion of the pre-spill microbial community. There are hundreds of species of bacteria, archaea, and fungi that can degrade petroleum.

Most petroleum hydrocarbons are biodegradable under aerobic conditions; though a few compounds found in crude oils, e.g. resins, hopanes, polar molecules, and asphaltenes, have practically imperceptible biodegradation rates. Lighter crudes, such as the oil released from the BP *Deepwater Horizon* spill, contain a higher proportion of simpler lower molecular weight hydrocarbons that are more readily biodegraded than heavy crudes, such as the oil released from the *Exxon Valdez*. The polycyclic aromatic hydrocarbons (PAHs) are a minor constituent of crude oils; however, they are among the most toxic to plants and animals. Bacteria can convert PAHs completely to biomass, CO<sub>2</sub>, and H<sub>2</sub>O, but they usually require the initial insertion of O<sub>2</sub> via dioxygenase enzymes. Anaerobic degradation of petroleum hydrocarbons can also occur albeit at a much slower rates. Petroleum hydrocarbons can be biodegraded at temperatures below 0°C to more than 80°C. Microorganisms require elements other than carbon for growth.



The concentrations of these elements in marine environments—primarily nitrates ( $\text{NO}_3^-$ ), phosphates ( $\text{PO}_4^{3-}$ ), and iron (Fe)—can limit oil rates of oil biodegradation. Having an adequate supply of these rate limiting nutrients when large quantities of hydrocarbons are released into the marine environment is critical for controlling the rates of biodegradation and hence the persistence of potentially harmful environmental impacts. Bioremediation, which was used extensively in the *Exxon Valdez* spill, involved adding fertilizers containing nitrogen (N) nutrients to speed up the rates of oil biodegradation.

Most petroleum hydrocarbons are highly insoluble in water. Hydrocarbon biodegradation takes place at the hydrocarbon-water interface. Thus the surface area to volume ratio of the oil can significantly impact the biodegradation rate. Dispersants, such as Corexit 9500, which was used during the BP *Deepwater Horizon* spill, increase the available surface area and, thus, potentially increase the rates of biodegradation.

**Overarching Differences Between the Two Spills**

Once the BP *Deepwater Horizon* oil leak started, the public and the popular media began to compare it to the *Exxon Valdez* spill which had been up until that time the largest marine spill in the United States. The public notoriety of *Exxon Valdez* spill was dramatic due to its impact on Alaska wildlife and the long litigation process, which is still seeing court action. However, the *Exxon Valdez* and BP *Deepwater Horizon* oil spills were vastly different in terms of the volume of oil, the nature of the oil, and the environments impacted (Table 1). The BP *Deepwater Horizon* oil spill was more than an order of magnitude greater in total volume of oil than the *Exxon Valdez* spill; the BP spill also released considerable amounts of natural gas (methane [ $\text{CH}_4$ ]). The *Exxon Valdez* spill occurred near shore and occurred as a surface slick, while the BP *Deepwater Horizon* spill was a leak from a well 5000 ft (1500 m) below the ocean surface as both a deep-sea “cloud” or “plume” and a surface water slick, more than 50 mi (80 km) from the nearest shore. The BP *Deepwater Horizon* spill was a light crude and more inherently biodegradable initially than the *Exxon Valdez* heavy crude from the North Slope of Alaska. The environments impacted were also very different in terms of climate, weather, and ecosystems, with the *Exxon Valdez* spill occurring in a sub-Arctic region and the BP *Deepwater Horizon* spill occurring in a sub-tropical region, although the deepwater region directly impacted by the BP spill was cold ( $<5^\circ\text{C}$ ). The Gulf of Mexico has lots of natural seeps of oil and there have been other spills from drilling rigs, such as the *IXTOC* well blowout of 1979. This is in contrast to the relatively pristine conditions of Prince William Sound which is much more enclosed and shallower than the more open ocean environment where the BP *Deepwater Horizon* spill occurred in the Gulf of Mexico. Indeed the treatments used were also quite different.

Since a storm with 50 mi/h (80 km/h) winds hit Prince William Sound within 2 days of the initial spill, no dispersant was used. Much of the oil washed onto the shorelines of islands in the path of the oil, making shoreline cleanup the primary focus. During the *Exxon Valdez* spill water washing and bioremediation (biostimulation using fertilizers containing N nutrients) were the major strategies. In the case of the BP *Deepwater Horizon* spill millions of gallons (1 U.S. gal = 3.79 L) of dispersant was used both on the surface and at the leaking wellhead in the Gulf of Mexico. A major focus was to protect shorelines from oil contamination. This was also the first time dispersant had been applied to a deepwater leaking well, primarily for safety reasons to prevent the highly flammable oil from reaching the surface immediately above the wellhead

where many ships were involved in leak operations. The BP *Deepwater Horizon* spill was the largest emergency response to a marine oil spill that the world has seen to date. In addition to dispersant, controlled burns, skimming, siphoning from the wellhead, containment booms, shoreline scavenging/berms and beach sand mixing were used extensively to mitigate the spill's impact.

Although numerous physical means were used to remove or disperse the oil, ultimately it was the microbes that played the major role in mitigating the environmental impacts of these two worst oil spills in U. S. history.

### **The Exxon Valdez Spill in Prince William Sound**

On March 24, 1989 the oil tanker *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, AK, spilling an estimated 11 million gallons (42 million liters) of crude oil that spread as a surface slick<sup>1</sup> (Figure 1). At the time this was the worst U. S. oil spill disaster. Dispersant tests were quickly conducted but due to weather conditions and the nature of the oil, which was a North Slope relatively heavy oil (API gravity=29), as well as State of Alaska concerns about the use of dispersants, the decision was made not to try to disperse the oil. Despite efforts to contain the spill, tidal currents and winds caused a significant portion of the oil to float ashore. Approximately 486 mi (778 km) of the 3000 mi (4800 km) of shoreline in Prince William Sound, and 818 mi (1309 km) of the 6000 mi (9600 km) of shoreline in the Gulf of Alaska, or about 15% of the total shoreline, became oiled to some degree<sup>2</sup>. Much of this oiling, especially in the Gulf of Alaska, was patchy and scattered in a light covering, e.g. as tar balls. Oiling was heaviest on the shorelines of islands in Prince William Sound that were directly in the path of the slick.

### **Assessing the efficacy and safety of bioremediation**

Because of the difficulty of achieving sufficient oil removal by physical washing and collection, especially for oil that had moved into the subsurface, bioremediation became a prime candidate for continuing treatment of the shoreline. Bioremediation had been independently identified as a potential emerging technology within weeks of the spill. Both the EPA and Exxon quickly began laboratory tests, which were soon followed by field trials to determine whether fertilizer addition would enhance the rates of oil biodegradation<sup>3,4</sup>. The focus of these tests was on the changes in oil composition due to microbial degradation, i.e. the emphasis was placed on changes in oil chemistry rather than on the microbes themselves.

Field tests showed that fertilizer addition enhanced rates of biodegradation by the indigenous hydrocarbon-degrading microorganisms. Rates of biodegradation in bioremediation studies resulted in total petroleum-hydrocarbon losses as high as 1.2% per day. The rate of biodegradation slowed down once the more readily degradable components were depleted even when fertilizer was reapplied. The rate of oil degradation was a function of the ratio of N/biodegradable oil and time. Both polynuclear aromatic and aliphatic compounds in the oil were extensively biodegraded. Bioremediation increased the rate of polycyclic-aromatic-hydrocarbon (PAH) degradation in relatively undegraded oil by a factor of 2, and of alkanes by 5 relative to the controls. O<sub>2</sub> dissolved in water was not rate-limiting—there was up to a 30%

decline in O<sub>2</sub> concentration in pore water following fertilizer application, but hypoxia was not detected.

**Full-scale use of bioremediation**

Based upon the laboratory and field demonstration test results, the federal on-scene-coordinator approved the use of bioremediation employing fertilizer application for use on oiled shorelines of Prince William Sound<sup>3,4,5</sup>. Several fertilizer formulations were considered; key considerations were retention in the oiled shorelines long enough to support biodegradation, availability in quantities needed to treat these shorelines, and lack of toxicity. Two fertilizers were selected for full scale bioremediation: the oleophilic fertilizer Inipol EAP22, manufactured by Elf Aquitaine of France; and the slow release fertilizer Customblen 28-8-0, manufactured by Sierra Chemicals of California. Customblen was spread at a rate of 27.8 g/m<sup>2</sup>. Inipol was then applied at a rate of 300g/m<sup>2</sup>. These rates ensured a safe margin below concentrations of ammonium (NH<sub>4</sub><sup>+</sup>) or NO<sub>3</sub><sup>-</sup> ions considered toxic by EPA water quality standards.

Results for sediment samples collected and analyzed in 1989 indicated that about 25-30% of the total hydrocarbon in the oil originally stranded on Prince William Sound shorelines had been lost within the first days to weeks after the spill. The natural background rates of oil biodegradation initially were estimated at 1.3 g oil/kg sediment/yr for surface oil and 0.8 g oil/kg sediment/yr for subsurface oil<sup>3</sup>. Concentrations of naturally occurring oil-degrading bacteria during this period were 1-5 x 10<sup>3</sup> cells/mL of seawater or about 1-10% of the total heterotrophic bacterial population. In late 1989 oil-degrading bacterial populations had greatly increased to about 1 x 10<sup>5</sup> cells/mL and made up about 40% of the heterotrophic population in oiled shoreline pore waters.

Large-scale applications of fertilizer during summer 1990 included over 1400 individual site treatments at 378 shoreline segments. Measurements in September of 1990 showed that the proportion of oil degrading bacteria had returned to background levels of under 1% of the total bacterial populations in pore waters. In 1991 about 220 individual site treatments were applied. By 1992 the length of shoreline still containing any significant amount of oil was 6.4 mi (10.2 km) or 1.3% of the shoreline oiled in 1989<sup>5</sup>.

In all, 107,000 lbs (48,600 kg) of N in the fertilizers were applied from 1989-1991, involving 2237 separate shoreline applications of fertilizer. This represents the largest use of bioremediation ever undertaken. A survey in May-June 1992 found that most of the oil had been removed from shorelines and on June 12, 1992 the U. S. Coast Guard and the State of Alaska officially declared the cleanup concluded. At that time some oil still remained but it was felt that further cleanup activities would not provide a net environmental benefit. The oil residue remaining in the shorelines was left to naturally biodegrade further although based upon previous oil spills it was clear that some residual oil would remain for an extended time period.

**Should bioremediation be reapplied today to treat residual subsurface oil?**

In 2001 and 2003 the National Oceanic and Atmospheric Administration (NOAA) conducted random sampling of 4,982 pits dug at 114 sites in Prince William Sound to determine how much residual oil remained<sup>6,7</sup>; these studies found that 97.8% of the pits had no oil or light oil residues even though these sites had been heavily-to-moderately oiled in 1989. Based upon the amount of



oil remaining as of 2001 it was estimated that there had been a 22% per year decline from 1991-2001 in the amount of oil remaining on the shore<sup>6</sup>. After 2001 the rates of decrease by natural processes of subsurface oil slowed to about 4% per year as the remaining oil became more weathered and more sequestered<sup>8</sup>. Additional grid surveys were conducted by ExxonMobil in 2002 and 2007<sup>9,10,11</sup> (Exxon having merged with Mobil in 1999). The 2007 survey at 22 sites reported to be heavily oiled in NOAA's 2001-2003 surveys found no oil in 71% of the pits, 21.8% had light levels or only traces of subsurface oil residue, 4.6% had moderate oiling and 2.6% had heavy oil levels<sup>9</sup>; 87% of the samples were completely depleted of alkanes and 82% also had lost more than 70% of the original PAHs<sup>12,13,14</sup>.

The residual oil occurs as localized patches. Persistent buried oil has been found in other spills, e.g. the *Florida* spill in a salt marsh in Falmouth, MA. In Prince William Sound the remaining oil residue is buried in boulder/cobble armored beaches in thin (typically about 10 cm thick) lenses containing fine-grained sediments. It is sequestered and the low water flow means that O<sub>2</sub> and nutrients found in the surrounding pore waters are not flowing through the oil layer, limiting biodegradation rates<sup>15,16</sup>, even though there are sufficient concentrations of nutrients and oxygen in the adjacent pore waters to support biodegradation of the residual oil components<sup>14</sup>. Most of the remaining subsurface oil residue is located in the mid-upper intertidal zone away from biota<sup>9,14</sup>. Concerns, however, have been raised that the lingering oil residue could have adverse impacts<sup>7,17,18</sup>. Given that the residual subsurface oil is sequestered the risks of mobilizing the oil through any treatment would seem to outweigh the potential benefits, i.e. the best approach would seem to simply allow the residual oil to slowly undergo further natural biodegradation. Nevertheless there have been proposals to bioremediate the remaining subsurface oil residues<sup>19,20</sup> even though direct exposure of biota has been demonstrated to be extremely unlikely<sup>21,22,23</sup>.

Venosa et al.<sup>20</sup> showed in laboratory experiments that if sediments were displaced, so that the oil was no longer sequestered, rapid biodegradation of the residual oil would occur. They concluded that O<sub>2</sub> is the main limiting factor. They also postulated that if NO<sub>3</sub><sup>-</sup> was added there could be anaerobic biodegradation of associated organic matter so that the porosity of the sediments would increase and oxygenated water could reach the oil. Given the patchy distribution of oil, the fact that most of the oil is already highly weathered so that the residual compounds are highly insoluble, and that sequestered oil is not reaching sensitive biota, Atlas and Bragg<sup>13</sup> have contended that the value of any such treatment will likely be very limited. Additional bioremediation field trials, though, are planned for 2011. The debate, thus, continues about whether bioremediation can still be effective more than 21 years after the spill.

### **The BP Deepwater Horizon Oil Leak in the Gulf of Mexico**

On April 20, 2010, high-pressure oil and gas escaped from BP's *Deepwater Horizon* exploratory well in Mississippi Canyon Block 252 which was located 77 km offshore. In the subsequent fire and explosions, 11 men tragically lost their lives. The *Deepwater Horizon* drilling rig burned and ultimately sank in 1500 m of water 2 days later. The blowout prevention device (BOP) at the wellhead and all the emergency shut-off equipment failed.

Upon sinking, the 21 inch (53 cm) riser pipe, from the wellhead to the drilling platform, collapsed onto the sea floor. Oil leaked from multiple locations along the riser pipe and the top of the BOP (Figure 2). In all, it took 84 days to stop the flow of oil from the *Deepwater Horizon* well. The

oil from this well (Macondo oil) is typical of light Louisiana crude from petroleum reservoirs more than 5000 m deep; it has an API gravity = 35.2<sup>24</sup>.

The actual volume of oil and gas released from the *Deepwater Horizon* well is very difficult to determine. The oil release was estimated at 4.9 million barrels (205.8 million gallons [780 million liters]) by the National Incident Command’s Flow Rate Technical Group (FRTG)<sup>24</sup>. Previously the *Ixtoc-I* well blowout in the Bay of Campeche, estimated at 147 million gallons (556 million liters), was the largest oil spill in the Gulf of Mexico and the second largest in the world (the largest spill was in the Persian Gulf in 1991 as a result of the intentional release of oil by Iraq). The Oil Budget calculator from the FRTG for the *Deepwater Horizon* well oil release estimated that 3% was skimmed, 5% was burned, 8% was chemically dispersed, 16% was naturally dispersed, 17% was captured, 25% was evaporated or dissolved, and 26% was remaining.

**Dispersion of oil.**

One of the strategies employed to defray the environmental and safety impact of the oil from the *Deepwater Horizon* was to inject the dispersant COREXIT 9500 directly at the wellhead or end of the riser pipe at a water depth of 1500 m. The goal was to disperse the oil at depth, thereby preventing large slicks from forming directly at the surface above the wellhead where many ships were gathered to stop the leak, and to prevent the oil from impacting the shoreline. The EPA established a rigorous, daily water sampling program, once it was demonstrated in early May that within 4 h of injecting COREXIT 9500 at the wellhead less oil was coming to the surface immediately above the wellhead making it safer for leak operations.

Additionally, there was physical dispersion because the oil was injected into the deep-sea at high pressure and temperature. While large oil droplets moved to the surface, droplets between 10-60 μm were neutrally buoyant and were picked up by the current between 900 - 1300 m<sup>25</sup>. The deep-water dispersed oil droplets that had a concentration of less than 10 ppm total petroleum hydrocarbons has been likened to a “cloud”. This “cloud” of dispersed oil could be detected by fluorescence moving away from the wellhead, generally in a southwesterly direction<sup>25</sup>. O<sub>2</sub> concentration drops that did not result in anoxic conditions often were also detected in association with the “cloud” of dispersed oil in the deep-sea<sup>26</sup>.

**Microbiology of the Deep-Sea “Cloud” of Dispersed Oil.**

The deep-sea “cloud” of dispersed oil was found to have lower PO<sub>4</sub><sup>3-</sup> and dissolved O<sub>2</sub> concentrations, slightly higher NH<sub>4</sub><sup>+</sup> concentrations and significantly lower NO<sub>3</sub><sup>-</sup> concentrations<sup>26</sup> suggesting bacterial activity in the “cloud” of dispersed oil. The total bacterial density was significantly higher in the “cloud” (up to 10<sup>5</sup> cells/mL) versus outside the “cloud” (approximately 10<sup>3</sup> cells/mL).

Using a 16S rRNA microarray, 951 subfamilies of bacteria were detected from 62 phyla; however, only 16 subfamilies of the γ-proteobacteria were significantly enriched in the cloud, with 3 families in the class *Oceanospirillales* dominating<sup>26</sup>. Clone libraries, qPCR, phospholipids, and functional gene arrays further supported the finding of enrichment of oil degraders. The “cloud community” was also cold loving (psychrophilic) since the temperature below 700 m in the Gulf of Mexico is always 2-5°C.

The average half-life of alkanes from two different cloud analyses and two different lab microcosm assays ranged from 1.2 – 6.1 days<sup>26</sup>, which are similar to those reported for other cold-water studies<sup>27</sup> (Table 2). During the release (April-July), concentrations of polynuclear aromatic hydrocarbons also decreased rapidly with distance from the release point (the wellhead) and were seen to reach <1.0 ppb within 15-20 mi (24-32 km) in all directions other than to the southwest, where a small number of samples exceeded 1 ppb out to 40 mi (64 km)<sup>28</sup>. Much of decline in PAHs is attributable to microbial degradation.<sup>29</sup>

Gaseous compounds also were biodegraded in the water column. Valentine et al.<sup>30</sup> reported that early in the spill propane (C<sub>3</sub>H<sub>8</sub>) and ethane (C<sub>2</sub>H<sub>6</sub>) were the primary drivers of microbial respiration, accounting for up to 70% of the observed oxygen dips in fresh “plumes.” Based on CH<sub>4</sub> and O<sub>2</sub> distributions Kessler et al.<sup>31</sup> reported that within ~120 days from the onset of release, a vigorous deepwater bacterial bloom of methanotrophs had respired nearly all the released methane. Molecular analyses for methanotrophs in September 2010 showed relative abundances of 5-36% of the gene sequences detected, whereas in June 2010, before the leak was stopped, no methanotrophs were detected. Clearly as the spill events progressed the microbial populations changed in response to the available hydrocarbons.

### **Oil biodegradation in surface waters and sediments**

There have been reports of sediment contamination based upon visual observations<sup>32</sup>. Sediment collected from more than 120 sites showed qualitative evidence for oil in up to 29% of the cores. However, detailed chemical analyses indicate that only 6% of these cores were contaminated with Macondo oil, all of which were within 2.7 km of the wellhead<sup>33</sup>. Thus, the evidence so far indicates that sediment contamination was limited primarily to near the wellhead.

With regard to surface oil and shorelines, up to 40% of the oil was lost in the water column between the wellhead and the surface, largely due to dissolution and mixing as the oil moved to the surface and evaporation as soon as it reached the surface which lowered the hydrocarbon concentrations and changed the composition of the oil<sup>34,35</sup>. Analyses of surface oil samples from the source toward the shore showed that volatile organic compounds were either dissolved or evaporated from the Macondo oil near the source, and oil that approached the near shore environment no longer had BTEX compounds present<sup>34,35,36</sup>. Photooxidation may also have been important for oil on the surface as it moved shoreward. In samples that were analyzed for BTEX, these compounds were never detected in Macondo oil that reached the shore, nor were BTEX compounds detected in near shore sediments<sup>33</sup>. Dissolution and evaporation appear to have been more important than biodegradation in the weathering of the surface slick<sup>34,36</sup>. Evaporation resulted in the loss of alkanes with chain lengths up to C<sub>20</sub>. Clearly physical dispersion and evaporation competed with biodegradation so that the overall weathering of the oil that did reach the shore was the result of multiple processes. Certainly the oil that has sunk into the shoreline and marsh sediments will degrade much more slowly as it becomes nutrient depleted and potentially anaerobic due to O<sub>2</sub> diffusion limitations. It is too soon to tell what the impact of the Macondo oil will be to the delicate marsh environments and beach communities in Louisiana, Mississippi, Alabama, and Florida, many further studies will be needed.

### **Summary**

The *Exxon Valdez* and BP *Deepwater Horizon* oil spills provide a number of lessons regarding the role of microbial biodegradation in determining the fate of the spilled oil. Biodegradation and other natural weathering processes will remove most of the contaminating hydrocarbons but it can take months to years in areas of high oil concentrations. Such was the case for oil on shorelines impacted by the *Exxon Valdez* oil spill. The major focus of biodegradation for the *Exxon Valdez* was on the shorelines—oil moved on the surface and while there were studies on decreasing concentrations of oil in the water column no specific biodegradation studies were conducted as they were for the BP spill with its unique deep-water cloud of dispersed oil. Also the advanced molecular techniques for characterizing microbial communities were not available at the time of the *Exxon Valdez* spill; given the advances in molecular biology over the past two decades it is not surprising, therefore, that extensive molecular analyses of microbial communities have been performed in the Gulf of Mexico following the BP *Deepwater Horizon* spill.

When oil is highly dispersed in the water column and where microbial populations are well adapted to hydrocarbon exposure, such as in Gulf of Mexico waters, biodegradation of oil proceeds very rapidly. Bioremediation through fertilizer addition can be an effective means of speeding up rates of oil biodegradation in some situations. One should, however, not expect 100% removal of oil by biodegradation—patches of highly weathered oil likely will remain in some environments. Decisions as to whether or not to rely upon microbial oil biodegradation, including whether to apply bioremediation, should be driven by risk and not just the presence of detectable hydrocarbons. In the case of the BP *Deepwater Horizon* spill, the leak was capped on July 15; by the first week of August, no surface oil slick was observed and concentrations of detectable oil in the water column were greatly diminished<sup>33</sup>. The natural rapid attenuation of oil in the BP *Deepwater Horizon* spill is due to a number of parameters, e.g. type of crude, offshore, jetting of the oil in to the deep-sea, rapid dissolution, and microbial adaption. The Gulf of Mexico has more natural seeps of oil than any marine area in North America, contributing more than 400,000 barrels of oil a year to the Gulf of Mexico<sup>37</sup>. In the Gulf of Mexico the microbiota are likely to be better adapted to oil because of natural seeps and offshore drilling then almost anywhere else in the world. Thus, it is not surprising that bacteria in the Gulf of Mexico responded rapidly to the influx of oil.

In conclusion, the fate of all oil spills will depend upon a unique set of circumstances that will govern risk and impacts, including the volume of oil spilled, the chemical nature of the oil, and the ecosystems with their specific environmental conditions impacted by the spilled oil. However, one common denominator is the cosmopolitan nature of oil-degrading microbes. Natural and enhanced biodegradation greatly reduced the concentrations of oil following both the *Exxon Valdez* and BP *Deepwater Horizon* oil spills. It was the unseen microbes that were largely responsible for the disappearance of the spilled oil that had spread into the environment. Responders to future spills would do well to mobilize as rapidly as possible a scientific understanding of the unique conditions of the spill, i.e. to determine both natural and enhanced biodegradation and what the best possible approach will be to minimize the risk and impact of the spill on the environment.

Literature Cited

1. Wolfe D. A.; Hameedi, M. J.; Galt, J. A.; Watabayashi, D.; Short J.; O'Clair, C.; Rice, S.; Michel, J.; Payne, J. R.; Braddock, J.; Hanna, S.; Sale, D. Fate of the oil spilled from the T/V Exxon Valdez in Prince William Sound, Alaska. *Environ. Sci. Technol.* **1994**, 28:561A–568A.
2. Owens, E. H. Shoreline conditions following the Exxon Valdez oil spill as of Fall 1990. In *Proceedings of the 14th Arctic and Marine Oilspill Program (AMOP) Technical Seminar*, Environment Canada, Vancouver, British Columbia, Canada, 1991; pp. 579-606.
3. Bragg, J. R.; Prince, R. C.; Wilkinson, J. B.; Atlas, R. *Bioremediation for Shoreline Cleanup Following the 1989 Alaskan oil Spill*. Exxon: Houston, TX, 1992.
4. Pritchard, P. H.; Costa, C. F. EPA's Alaska oil spill bioremediation project. *Environ. Sci. Technol.* **1991**, 25, 372–379.
5. Bragg, J. R.; Prince, R. C.; Harner, E. J.; Atlas, R. M. Effectiveness of bioremediation for the Exxon Valdez oil spill. *Nature* **1994**, 368, 413–418.
6. Short, J. W.; Lindeberg, M. R.; Harris, P. M.; Maselko, J. M.; Pellak, J. J.; Rice, S. D. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the Exxon Valdez oil spill. *Environ. Sci. Technol.* **2004**, 38, 19–25.
7. Short, J. W.; Maselko, J. M.; Lindeberg, M. R.; Harris, P. M.; Rice, S. D. Vertical distribution and probability of encountering intertidal Exxon Valdez oil on shorelines of three embayments within Prince William Sound. *Environ. Sci. Technol.* **2006**, 40, 3723–3729.
8. Short, J. W.; Irvine, G. V.; Mann, D. H.; Maselko, J. M.; Pella, J. J.; Lindeberg, M. R.; Payne, J. M.; Driskell, W. B.; Rice, S.D. Slightly weathered Exxon Valdez oil persists in Gulf of Alaska beach sediments after 16 Years. *Environ. Sci. Technol.* **2007**, 41, 1245-1250.
9. Boehm, P. D.; Page, D. S.; Brown, J. S.; Neff, J. M.; Bragg, J. R.; Atlas, R. M. Distribution and weathering of crude oil residues on shorelines 18 years after the Exxon Valdez spill. *Environ Sci Technol.* **2008**, 42, 9210–9216.
10. Owens, E. H.; Taylor, E.; Humphrey, B.. The persistence and character of stranded oil on coarse-sediment beaches. *Mar. Pollut. Bull.* **2008**, 56, 14-26.
11. Taylor, E.; Reimer, D. Oil persistence on beaches in Prince William Sound – A review of SCAT surveys conducted from 1989 to 2002. *Mar. Pollut. Bull.* **2008**, 43, 458-474.
12. Atlas, R. M.; Bragg, J. Assessing the long-term weathering of petroleum on shorelines: uses of conserved components for calibrating loss and bioremediation potential. In *Proceedings of the 29th Arctic and Marine Oil Spill Program (AMOP)*. June 5-7, Edmonton, Alberta, Canada. 2007, pp. 263-290.

13. Atlas, R.; Bragg, J. R. Bioremediation of marine oil spills: when and when not - the *Exxon Valdez* experience. *Microbial Biotechnol.* **2009**, *2*, 213–221.
14. Atlas, R.; Bragg, J. R. Evaluation of PAH depletion of subsurface *Exxon Valdez* oil residues remaining in Prince William Sound in 2007-2008 and their likely bioremediation potential. In *Proceedings of the 32nd Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*; Environment Canada: Ottawa, ON, **2009**; Vol. 2, pp 723-747.
15. Boufadel, M, C.; Harifi, Y.; VanAken, B.; Wrenn, B.; Lee, K. Nutrient and oxygen concentrations within the sediments of an Alaskan beach polluted with the *Exxon Valdez* Oil Spill. *Environ. Sci. Technol.* **2010**, *44*, 7418–7424.
16. Li, H.; Boufadel, M.C. Long-term persistence of oil from the Exxon Valdez spill in two-layer beaches. *Nat. Geosci.* **2010**, *3*, 96–99.
17. Bodkin, J. L.; Ballachey, B. E.; Dean, T. A.; Fukuyama, A. K.; Jewett, S. C.; McDonald, L.; Monson, D. H.; O’Clair, C. E.; VanBlaricom, G. R. Sea otter population status and the process of recovery from the 1989 *Exxon Valdez* oil spill. *Mar. Ecol. Prog. Ser.* **2002**, *241*, 237–253.
18. Esler, D.; Bowman, T. D.; Trust, K. A.; Ballachey, B. E.; Dean, T. A.; Jewett, S. C.; O’Clair, C. E. Harlequin duck population recovery following the Exxon Valdez oil spill: Progress, process and constraints. *Mar. Ecol. Prog. Ser.* **2002**, *241*, 271–286.
19. Michel, J.; Nixon, Z.; Cotsapas, L. *Evaluation of oil remediation technologies for lingering oil from the Exxon Valdez oil spill in Prince William Sound, Alaska*. The Exxon Valdez Oil Spill Trustee Council Restoration Project No. 050778 Final Report. 2006.
20. Venosa, A. D.; Campo, P.; Suidan, M. T. Biodegradability of lingering crude oil 19 years after the *Exxon Valdez* oil spill. *Environ. Sci. Technol.* **2010**, *44*, 7613–7621.
21. Boehm, P. D.; Neff, J. M.; Page, D. S. Assessment of polycyclic aromatic hydrocarbon exposure in the waters of Prince William Sound after the Exxon Valdez oil spill: 1989–2005. *Mar. Pollut. Bull.* **2007**, *54*, 339–356.
22. Boehm, P. D.; Page, D. S.; Neff, J. M.; Johnson, C. B. Potential for sea otter exposure to remnants of buried oil from the Exxon Valdez oil spill. *Environ. Sci. Technol.* **2007**, *41*, 6860–6867.
23. Neff, J. M.; Bence, A. E.; Parker, K. R.; Page, D. S.; Brown, J. S.; Boehm, P. D. Bioavailability of polycyclic aromatic hydrocarbons from buried shoreline oil residues 13 years after the Exxon Valdez oil spill: A multispecies assessment. *Environ. Toxicol. Chem.* **2006**, *25*, 947–961.
24. The Federal Interagency Solutions Group: Oil Budget Calculator Science and Engineering Team. 2010. Oil Budget Calculator Technical Documentation.



[http://www.restorethegulf.gov/sites/default/files/documents/pdf/OilBudgetCalc\\_Full\\_HQ-Print\\_111110.pdf](http://www.restorethegulf.gov/sites/default/files/documents/pdf/OilBudgetCalc_Full_HQ-Print_111110.pdf).

25. Camilli, R.; Reddy, C. M. ; Yoerger, D. R. ; Van Mooy, B. A. S. ; Jakuba, M. V. ; Kinsey, J. C.; McIntyre, C. P. ; Sylva, S. P. ;Maloney, J. V.. Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. *Science* **2010**, *330*, 201-204.

26. Hazen, T. C.; Dubinsky, E. A.; DeSantis, T. Z.; Andersen, G. L.; Piceno, Y. M.; Singh, N.; Jansson, J. K.; Probst, A.; Borglin, S. E.; Fortney, J. L.; Stringfellow, W. T.; Bill, M.; Conrad, M. E.; Tom, L. M.; Chavarria, K. L.; Alusi, T. R.; Lamendella, R.; Joyner, D. C.; Spier, C.; Baelum, J.; Auer, M.; Zemla, M. L.; Chakraborty, R.; Sonnenthal, E. L.; D'Haeseleer, P.; Holman, H. Y. N.; Osman, S.; Lu, Z. M.; Van Nostrand. J.; Deng, Y.; Zhou, J. Z.; Mason, O. U. Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science* **2010**, *330*, 204-208.

27. Venosa, A. D.; Holder, E. L. Biodegradability of dispersed crude oil at two different temperatures. *Mar. Pollut. Bull.* **2007**, *54*, 545-553.

28. Boehm, P. D.; Cook, L.; Murray, K. J. Aromatic hydrocarbon concentration in seawater: Deepwater Horizon oil spill. In *Proceedings 2011 International Oil Spill Conference*. American Petroleum Institute: Washington DC, 2011.

29. Boehm, P. D., L. L. Cook, R. Barrick, and R. M. Atlas. Preliminary water column PAH exposure assessment: weathering of Oil in the water column, and evidence for rapid biodegradation. Paper presented at Gulf Oil Spill Focused Topic SETAC Meeting. Pensacola Florida. April 26, 2011.

30. Valentine, D. L.; Kessler, J. D.; Redmond, M. C. ; Mendes, S. D.; Heintz, M. B.; Farwell, C.; Hu, L.; Kinnaman, F. S. ; Yvon-Lewis, S. ; Du, M. R. ; Chan, E. W.; Tigreros, F. G.; Villanueva, C. J. Propane respiration jump-starts microbial response to a deep oil spill. *Science* **2010**, *330*, 208-211.

31. Kessler, J. D.; Valentine, D. L.; Redmond, M. C.; Du, M. R.; Chan, E. W.; Mendes, S. D.; Quiroz, E. W.; Villanueva, C. J.; Shusta, S. S.; Werra, L. M.; Yvon-Lewis, S. A.; Weber, T. C. A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico. *Science* **2011**, *331*, 312-315.

32. Joye, S. Offshore ocean aspects of the Gulf oil well blowout. Paper Presented at AAAS Annual Meeting, Washington DC. Feb. 19, 2011.

33. Operational Science Advisory Team. 2010. Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Sampling and Monitoring. New Orleans: Unified Area Command. [http://www.restorethegulf.gov/sites/default/files/documents/pdf/OSAT\\_Report\\_FINAL\\_17DEC.pdf](http://www.restorethegulf.gov/sites/default/files/documents/pdf/OSAT_Report_FINAL_17DEC.pdf).

34. Gong, C.; Milkov, A.V.; Grass, D.; Sullivan, M.; Searcy, T.; Dzou, L.; Depret, P. The significant impact of weathering on MC252 oil chemistry and its fingerprinting of samples

collected from May to September 2010, Paper presented at SETAC North America 31st Annual Meeting, Portland, OR, November 7-11, 2010.

35. Operational Science Advisory Team. 2011. Summary Report for Fate and Effects of Remnant Oil in the Beach Environment. New Orleans: Gulf Coast Incident Management Team. <http://www.restorethegulf.gov/sites/default/files/u316/OSAT-2%20Report%20no%20ltr.pdf>.

36. Brown, J. S. ; Beckmann, D.; Bruce, L.; Mudge, S. PAH depletion ratios document rapid weathering and attenuation of PAHs in oil samples collected after the Deepwater Horizon Incident. Paper presented at SETAC North America 31st Annual Meeting, Portland, OR, November 7-11, 2010.

37. *Oil in the Sea III: Inputs, Fates, and Effects*; National Academy of Sciences: The National Academies Press, Washington, DC, 2003.

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Table 1. Comparison of BP Deepwater Horizon and *Exxon Valdez* Spills

<i>Exxon Valdez</i>	BP Deepwater Horizon
41.6 million liters	779 million liters estimated by the National Incident Command's Flow Rate Technical Group (FRTG)
North Slope Heavy Oil (API 29)	Light Louisiana Oil (API 35.2)
Tanker spill with known volume	Well leak with uncertain flow rates, large amounts of methane also released
Discharged as surface spill	Discharged at well head in 1500m of seawater
On Bligh (Near Island shorelines)	77 km offshore
Impacted cobble/rocky shorelines, major storm in area with 50 mph winds 2 days after spill	Impacted deep-sea cloud of fine droplets of low concentration oil, marshes and sandy beach shorelines, 84 days to stop leak
Bioremediation used extensively	Aerial and subsurface dispersants used extensively
Fate of oil remnants still studied more than 21 years after spill	Fate of oil remnants yet to be determined
Much scientific and operating experience gained is applicable to other spills	Largest remediation and emergency response to an oil spill ever, worldwide

Table 2. Oil Biodegradation Half-Life Comparisons

Crude Type	Source	Half-Life (days)	Reference
Light Crude (API35)	Macondo Oil (MC252 block)	1.2-6.1 alkanes <i>in situ</i> change; 2.2-3.5 alkanes microcosm, enriched consortia	26
Heavy Crude (API28)	Prudhoe Bay	4.6 alkanes, enriched consortia with dispersant; 9.9 alkanes, enriched consortia without dispersant	27

Figure 1. Graphic depiction of *Exxon Valdez* spill and cleanup.

Figure 2. Graphic depiction of Deepwater Horizon spill and cleanup.